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Severe Neutropenia Associated With IgG2 Subclass Deficiency and Bone Marrow T-Lymphocyte Infiltration

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Patients with selective IgG2 subclass deficiency (IgG2 SD) usually suffer from recurrent respiratory infections. The occurrence of cytopenia is extremely rare in these patients. We report on two patients with isolated IgG2 SD who experienced unexplained severe neutropenia associated with T-lymphocyte proliferation. IgG2 SD clearly preceded the occurrence of neutropenia in one patient. In the other patient, the long-standing history of recurrent respiratory infections prior to diagnosis of agranulocytosis suggests that IgG2 SD also preceded the occurrence of neutropenia. Analysis of bone marrow biopsy in both patients and skin tissue lesions in one patient showed massive infiltration with CD4+ and CD8+ T-lymphocytes. The pathological feature did not suggest any malignant lymphoproliferative disorder. Neutropenia was refractory to i.v. Ig in both patients and to recombinant G-CSF, steroids, and cyclophosphamide in one patient. Severe cellulitis led to death in one patient. In summary, we reported herein a heretofore undescribed syndrome characterized by the association of IgG2 SD with severe neutropenia and tissue T-cell infiltration. It suggests that bone marrow analysis as well as determination of serum IgG subclasses need to be performed in patients with unexplained neutropenia. Am. J. Hematol. 57:241–244, 1998.

Key words: immunodeficiency; IgG2 subclass deficiency; neutropenia; lymphoproliferation

INTRODUCTION

IgG2 subclass deficiency (SD) can be either isolated or associated with other primary immune deficiencies such as IgA deficiency and Wiskott Aldrich syndrome [1–4]. Patients with selective IgG2 SD usually suffer from frequent upper and lower respiratory tract infections [1,2]. In rare cases, they may develop autoimmune neutropenia [5], vasculitis [6], or intractable epilepsy [7]. However, to our knowledge lymphoid proliferation has not been reported in patients with selective IgG2 SD. We report on two patients with IgG2 SD who developed severe neutropenia and T-lymphocyte proliferation.

PATIENTS AND METHODS

Two patients were referred to the Immunology and Hematology Department of Hôpital Saint-Louis (Paris, France) because of severe neutropenia.

Serum IgG, IgA, and IgM levels were evaluated using a rate nephelometry method (Arrey Protein System, Beckman, Palo Alto, CA). Normal values were IgG: 9.95 (±3.50) g/L; IgA: 1.95 (±1.25) g/L; IgM: 2.0 (±1.45) g/L. Serum IgG subclasses levels were determined using an ELISA method [8]. Normal values in adult were as follows: IgG1: 7.55 (±2.45) g/L; IgG2: 3.0 (±1.50) g/L; IgG3: 0.50 (±0.20) g/L; IgG4: 0.55 (±0.5) g/L. Immunofixation was used to detect serum monoclonal immunoglobulin [9,10]. Anti-granulocyte autoantibodies were detected by indirect immunofluorescence [11,12] and using an in vitro microagglutination test [13].

Analysis of bone marrow myeloid progenitors growth was performed as previously described [14].

Lymphocyte immunophenotyping was performed on heparinized blood by immunofluorescence with the com-

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mercial available monoclonal antibodies (mAbs) to CD3, CD4, CD8, and CD19 (Becton Dickinson, San Jose, CA). Immunological markers were analysed on a FACScan flow cytometer (Becton Dickinson).

On tissue sections, B cells were identified by the use of mAbs to CD19 and CD20, and T cells using anti-CD3, CD4, and CD8 mAbs (Dako, Carpinteria, CA).

Blood lymphocyte clonality was assessed by PCR amplification of VH-JH rearrangements using FR1 family specific as well as FR3 primers and a JH consensus primer as previously described [15].

PATIENTS AND RESULTS

Patient 1 is a 19-year-old male with a medical history of frequent upper respiratory tract infections since childhood. When he was 18, he developed scrotal necrosis and omentum infarction without evidence for vasculitis on biopsies. There was no spleen, liver or lymph node enlargement. He also developed profound leucopenia (leucocytes $<0.5 \times 10^9$/L) with severe neutropenia (neutrophils $<0.1 \times 10^9$/L), which was associated with recurrent severe infections. Serum gammaglobulins were slightly decreased: 6.80 ± 0.20 g/L (normal: 9–12 g/L). IgG2 serum levels were consistently low (0.35 ± 0.05 g/L) and serum IgG1, IgG3, IgG4, IgM, and IgA levels were normal. Analysis of bone marrow (Fig. 1A) and scrotal skin biopsies (not shown) disclosed a sparse lymphocytic infiltration as well as focal aggregates of small lymphocytes with slightly irregular nuclei and scant cytoplasm. In the bone marrow, these nodules were preferentially centromedullar or in some areas paratrabecular. Immunohistochemical analysis indicated that most lymphocytes were T cells (CD3+) of both CD4 and CD8 immunophenotype. No clonal B- or T-cell population was detected by PCR in the peripheral blood.

Autoantibodies to polymorphonuclear neutrophils (PN) with unidentified specificity were present in the patient serum. Neutropenia was refractory to high-dose corticosteroids, cyclophosphamide, i.v. immunoglobulins (0.4 g/kg/day for 5 days), and granulocyte-colony stimulating factor (up to 15 mg/kg/d for 1 month). After 1-year follow-up, the patient developed severe abdominal cellulitis and died.

Patient 2 is a 43-year-old woman with a long-standing history of eczema, splenomegaly, recurrent fever, and facial microvesicular eruption due to herpes simplex virus (HSV). Lymph nodes and liver were not enlarged. Her blood neutrophil counts had been within normal range up to age 27 and progressively declined reaching 0.5×10^9/L by age 43. Repetitive bone marrow aspiration analysis disclosed a decrease in myeloid lineage cells without maturation arrest. A relative excess of lymphocytes (25–45%) could be detected since age 28. Bone marrow biopsy displayed T lymphocyte infiltration similar to that seen in patient 1 (Fig. 1B). In vitro bone marrow cell culture demonstrated normal CFU-GM formation at day 10, reaching a maximum at day 15. No inhibitory effect of patient's serum on the myeloid precursor cell growth was detected. Serum gammaglobulin level was 8.80 ± 0.25 g/L including consistent low IgG2 concentration (0.51 ± 0.07 g/L) but normal IgG1, IgG3, IgG4, IgM, and IgA. Analysis of stored frozen serum samples indicated that low IgG2 levels were present up to 12 years before occurrence of neutropenia. Circulating B-cell and T-cell subpopulations were as follows: CD19+: 24×10^6/L, CD3+: 501×10^6/L including CD4+: 294×10^6/L and CD8+: 204×10^6/L. CD4+ T lymphocytes included 49% CD28+ cells. Among CD8+T cells 24% were CD28+ and 11% CD57+. CD16+CD56+ cells constituted 6% of total circulating lymphocytes. Immunoglobulin and T-cell receptor gene analysis did not reveal any clonal population in peripheral blood lymphocytes. In vitro proliferation of peripheral blood lympho-

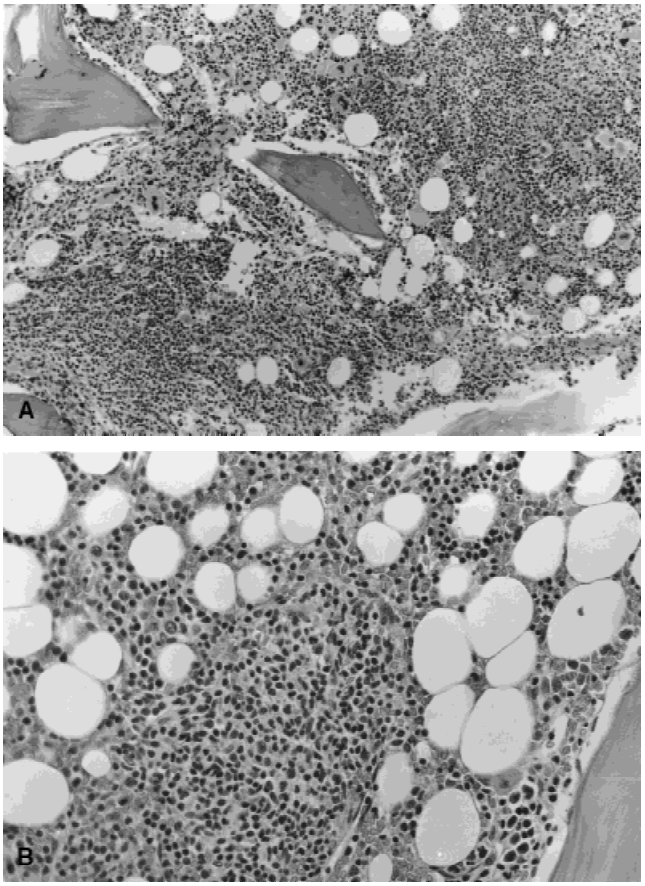

Fig. 1. Bone marrow analysis of patient 1 (A) and patient 2 (B). A: Bone marrow biopsy shows a dense lymphocytic infiltration forming ill-delimited centromedular nodules in nearly each marrow space (magnification ×100). B: Location and density of the lymphocytic infiltrate is similar to that described in patient 1. Nodules are composed of small lymphocytes with slightly irregular nuclei (magnification ×250).

cytes was normal in the presence of mitogens (phytohemagglutinin, concanavalin A, pokeweed mitogen) and all the tested antigens (including tuberculin, candidin, and tetanoid anatoxin antigens) except HSV. PN count remained low under replacement treatment with i.v. gammaglobulins (0.4 g/kg/d every 4 weeks).

In both patients, hemoglobin levels and platelet counts were normal and no large granular lymphocyte could be detected in the peripheral blood. Autoantibodies, including rheumatoid factor and anti-nuclear antibodies, were negative with the exception of anti-PN in patient 1. There was no monoclonal immunoglobulin in the patients' sera. Both patients were hepatitis B virus, hepatitis C virus, HIV1-2, and HTLV1 antibody negative. They were not receiving myelotoxic drug.

DISCUSSION

Imbalances of IgG subclasses, including IgG2 are extremely common in primary immune deficiencies such as IgA deficiency, Ataxia Telangiectasia, or Wiskott-Aldrich syndrome [1,2].

Patients with selective IgG2 SD mainly suffer from recurrent respiratory tract infections including bronchitis and bronchopneumonia that may lead to bronchiectasia and obstructive lung diseases. Encapsulated bacteria, including haemophilus type b and streptococcus pneumoniae are common pathogens in these patients, reflecting an impaired antibody response to polysaccharides [16]. Up to 12.5% of infected IgG2 SD patients develop skin and/or visceral vasculitis including Henoch-Schönlein purpura [6]. Intractable epilepsy of childhood was also found to be associated with IgG2 SD [7]. Bussell et al. [5] mentioned the possibility that moderate IgG2 SD may associate in some patients with immune thrombocytopenic purpura or neutropenia. However, the pathogenesis of such manifestations remains unclear.

We report herein on two patients who developed low IgG2 serum levels associated with severe neutropenia and diffuse T-cell infiltration. Although diagnosis of IgG2 SD and neutropenia were simultaneously made in both patients, the long medical history of recurrent respiratory infections suggests that IgG2 SD was likely present for several years. Moreover, in patient 2 blood cell count has been normal for more than three decades while she was experiencing frequent respiratory infections. In this patient, IgG2 SD was retrospectively diagnosed in stored sera up to 12 years before the occurrence of neutropenia. The pathogenesis of neutropenia in patients with IgG2 SD is poorly understood as it is in other primary immunodeficiencies, including CVID, hyper-IgM syndrome, or IgG1 SD [17,18]. An auto-immune mechanism could be suggested in patient 1, in whom anti-neutrophils autoantibodies were detected. However, he did not respond to steroids or immunosuppressive therapy. A direct cytotoxic or suppressive effect of T lymphocytes infiltrating the bone marrow may also be suggested.

Follicular lymphoid hyperplasia and non Hodgkin lymphoma occur at a higher frequency in patients with primary immunodeficiencies including common variable immunodeficiency and hyper IgM syndrome [17,19–23]. Cyclic neutropenia associated with excess of polyclonal CD8+ T lymphocytes in both bone marrow and peripheral blood has been reported in a patient with severe hypogammaglobulinemia [24]. However, lymphoproliferation has so far not been reported in patients with selective IgG2 SD. Strikingly, our patients developed bone marrow and in one case skin T-cell infiltration. The pathological feature did not suggest any malignant lymphoproliferative disorder nor follicular hyperplasia. Lymph nodes were not clinically involved and in one patient the presence of splenomegaly might suggest spleen infiltration. The presence of both CD4+ and CD8+ lymphocytes suggests that the T-cell population within the tissue infiltrates is polyclonal, although clonality of these cells was not assessed by molecular biology. It is worth noting that no clonal T or B cells could be detected in the peripheral blood of both patients. Here again, the pathogenesis of the lymphocytic infiltration remains unclear. One might speculate that in a subset of patients with IgG2 SD, local hypersecretion of some T-cell chemoattractant cytokines could be responsible for such infiltrates. Another possibility could be a defect in T-cell apoptosis in the infiltrated tissues.

Our report points to a unique association of neutropenia and tissue T-cell infiltration in patients with IgG2 SD. It, therefore, suggests that serum IgG subclass levels must be determined in patients with unexplained severe neutropenia, particularly in those who have a medical history suggesting a defect in humoral immunity but whose total serum IgG, IgM, and IgA are normal.

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